

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 4, line 9 and ending on line 15 with the following rewritten paragraph:

-- However, all of the aforementioned labeling methods suffer from the inherent steric interference introduced by the size of the tag, typically larger than 100 D<sup>3</sup>, primarily contributed by the reporter group, usually an enzyme. By contrast, the antibiotic usually being a substantially smaller molecule (20 D<sup>3</sup>) than the macro-molecular complexes described above, can readily penetrate ~~membrane-bound~~ membrane-bound receptors on the cell surface. Consequently, a continuing need exists for a sensitive and rapid method to detect extremely small amounts of target biological analytes. --

Please replace the paragraph beginning on page 5, line 27 and ending on line 29 with the following rewritten paragraph:

-- Non-lantibiotic ~~bacteriocins~~, bacteriocins, such as plantaricin, thermophilin and mesentericin Y, may also be useful. Any of the ribosomally synthesized cationic defense proteins that bind to the membranes of target bacteria are included within the present claims. --

Please replace the paragraph beginning on page 6, line 1 and ending on line 12 with the following rewritten paragraph:

-- The invention also includes a method for synthesizing a bacteriocin-metal complex, comprising: (a) admixing (i) a water soluble salt of metal selected from the group consisting of transition metals and lanthanides with (ii) a bacteriocin selected from the group consisting of lantibiotics, non-lanthionine containing peptides, large heat labile proteins and complex bacteriocins, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof in (iii) a solvent for the metal salt and the bacteriocin, wherein the admixing is conducted under conditions effective

to promote chelation of the metal by the bacteriocin, thereby forming a solution of the complex of the bacteriocin and the metal; (b) desalting the complex; and (c) isolating and drying the complex. A particular advantage of these complexes is their ability to bind to viable cells, but not to nonviable cells, which allows the bacteriocin metal complexes to distinguish between viable cells and non-viable cells or cellular debris. --

Please replace the paragraph beginning on page 6, line 21 and ending on line 28 with the following rewritten paragraph:

-- When the bacteriocin-metal complex is allowed to bind to pathogens *in situ* in or on a sample, a portion of the pathogens present with the bound bacteriocin-metal complex is preferably removed for detection of pathogens, for example, by washing or using a swab or sponge, ~~for example~~. If using a swab, any pathogens that are present in or on the sample are removed from the swab and suspended in aqueous buffer solution. The number of organisms present in the buffer is determined by measuring the luminescence in the presence of an oxidizable substrate (e.g., luminol) and a source of peroxide. --

Please replace the paragraph beginning on page 7, line 27 and ending on page 8, line 5 with the following rewritten paragraph:

-- The invention also includes a therapeutic treatment comprising a bacteriocin-metal chelated complex comprised of (a) a bacteriocin selected from the group consisting of lantibiotics, [[,]] non-lanthionine containing peptides, large heat labile proteins and complex bacteriocins, fusion proteins thereof, mixtures thereof, and fragments, homologs and variants thereof, and (b) a detectable label comprising a transition or lanthanide metal, wherein injured or diseased tissue is treated with the bacteriocin-metal complex. A preferred transition metal is cobalt. A preferred lantibiotic is nisin. --

Please replace the paragraph beginning on page 11, line 3 and ending on line 20 with the following rewritten paragraph:

-- The present invention thus also includes within its scope bacteriocin homologs encoded by DNA sequences capable of hybridizing, preferably under stringent conditions, with the DNA sequences described herein, or sequences which code for the bacteriocin amino acid sequences disclosed herein using the degeneracy of the genetic code and coding for proteins having substantially the same activity. Stringent hybridization conditions select for DNA sequences of greater than 85% or, more preferably, greater than about 90% homology. Screening of a cDNA library may be carried out under highly stringent conditions according to the method described in European Patent Application No. 88 119 602.9 and Kashima et al. (Nature 313:402-404 (1985)). The DNA sequences capable of hybridizing under stringent conditions with the DNA sequences disclosed in the present application may be, for example, allelic variants of the disclosed DNA sequences, may be naturally present in the particular microorganism but related to the disclosed DNA sequences, or may be derived from other sources. General techniques of nucleic acid hybridization are disclosed by Maniatis, T. et al., [[in:]] Molecular Cloning, [[.]] a Laboratory Manual, Cold Spring Harbor, NY (1982), and by Haymes, B.D. et al., [[In:]] Nucleic Acid Hybridization, a Practical Approach, IRL Press, Washington, DC (1985), and by Sambrook, J. and Russell, D.W., [[In:]] Molecular Cloning, A Laboratory Manual, 3<sup>rd</sup> Edition, Cold Spring Harbor, NY (2001). --

Please replace the paragraph beginning on page 12, line 24 and ending on line 26 with the following rewritten paragraph:

-- The term "bacterial pathogen" refers to any microorganism known to induce a disease in an animal, such as gram positive bacteria, gram negative bacteria, mycobacteria, and the like . --

Please replace the paragraph beginning on page 12, line 27 and ending on line 29 with the following rewritten paragraph:

-- The term "non-bacterial pathogen" refers to fungi, viruses, prions, and the like without restriction. However, it is preferred that the bacteriocin metal complex binds to these pathogenic agents with an affinity constant of at least about  $10^8 M$  . --

Please replace the paragraph beginning on page 14, line 9 and ending on line 15 with the following rewritten paragraph:

-- It has been discovered that bacteriocins form a complex with ~~redox-active~~ redox-active metals requiring minimal chemical modification, and yield chemiluminescent bacteriocin-metal chelates. These bacteriocin-metal chelates appear to be as catalytically active as the oxidative enzymes and organo-metallic complexes of the porphyrins in catalyzing the hydrogen ~~peroxide-mediated~~ peroxide-mediated oxidation of luminol. Most significantly, these chelates are fully biologically active and are not sterically hindered by large enzymes or conjugated organic groups. --

Please replace the paragraph beginning on page 18, line 14 and ending on line 30 with the following rewritten paragraph:

-- While lantibiotics are the preferred bacteriocins, any of the generally cationic peptides synthesized by bacteria, plants, mammals or insects having antimicrobial activity and forming complexes with transition or lanthanide metals could be used. Therefore, diverse species of cationic membrane active peptides such as the non-lanthionine containing bacteriocins, defensins, cecropins, and magainins, for example, are equally

useful to generate metal complexes which bind to the membranes of pathogens, and can be used for the detection of pathogenic species. Fusion proteins, fragments, homologs and variants of these cationic peptides also are encompassed within the present invention, so long as membrane binding activity is preserved. However, the function of pore formation is not necessary for detection, and therefore, the bacteriocins or other cationic antimicrobial peptides, fusion proteins thereof, fragments, homologs and variants thereof are included even if the pore forming activity has been lost due to changes in amino acid sequence or secondary structure. Preferably, the present invention is applicable to any bacteriocin capable of binding to gram positive bacteria, mycobacteria, gram negative ~~bacteria~~ bacteria, and fungi. Gram positive bacteria are preferred targets for the bacteriocin-metal complexes of the present invention. In certain embodiments, permeabilized gram negative bacteria and fungi may be targeted. --

Please replace the paragraph beginning on page 42, line 24 and ending on page 43, line 3 with the following rewritten paragraph:

-- Bacteria were diluted in sterile 0.1% peptone from cell concentrations of  $10^7$  CFU/mL to 10 CFU/mL. The cells were treated with the Nisin-Co (II) complex (of Example 1 [[3]]) at 30  $\mu$ g/mL for twenty minutes at room temperature. The cells were collected by filtration on 0.2  $\mu$ m polycarbonate track etch membrane (Osmonics), rinsed with 1.0 mL peptone; after which the membrane was transferred to 1.5 mL micro-centrifuge tube. Chemiluminescence was measured using 0.2mL of Luminol reagent purchased from NEN Life Sciences (Boston, MA) in a Lum-T® luminometer. Figure 5 shows the titration curve for the cells. From the data presented, the lowest detectable cell concentration is estimated to be about 100 cells per sample. --